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Keywords
biomass, bole position, clones, correlation, feedstock quality, Populus, short rotation woody crops, tree growth, water stress, wood density

Disciplines
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Article

Specific Gravity of Hybrid Poplars in the North-Central Region, USA: Within-Tree Variability and Site × Genotype Effects

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Abstract: Specific gravity is an important consideration for traditional uses of hybrid poplars for pulp and solid wood products, as well as for biofuels and bioenergy production. While specific gravity has been shown to be under strong genetic control and subject to within-tree variability, the role of genotype × environment interactions is poorly understood. Most specific gravity reports are for a limited number of locations, resulting in a lack of information about the interactions between clones and sites over a wide range of climate and soil conditions. The objective of the current study was to characterize the effects of bole position, site, clone, and site × clone interactions for twelve hybrid poplar genotypes grown in Iowa, Minnesota, Wisconsin, and Michigan, USA. Observed specific gravities ranged from 0.267 to 0.495 (mean = 0.352 ± 0.001 for 612 samples taken from 204 trees), with bole position and site × clone interactions having significant effects on specific gravity. Further investigation of the site × clone interactions indicated that environmental conditions related to water stress were key predictors of specific gravity. These data are

important for informing genotypic selection and silvicultural management decisions associated with growing hybrid poplars.

Keywords: biomass; bole position; clones; correlation; feedstock quality; *Populus*; short rotation woody crops; tree growth; water stress; wood density

1. Introduction

Short rotation woody crops (SRWCs) such as *Populus* species and their hybrids (hereafter referred to as hybrid poplars) can be grown in strategic locations across the landscape to increase ecosystem services and maintain regional ecological sustainability [1]. These purpose-grown feedstock production systems provide regulating, provisioning, cultural, and supporting services as outlined in the Millenium Ecosystem Assessment [2], which are especially important for contributing benefits such as erosion control, soil health maintenance, and environmental remediation (*i.e.*, the regulating services). For example, certain *Populus* hybrids are used as components of agronomic intercropping systems to stabilize soils and provide critical wildlife habitat [3,4]. Hybrid poplars are also used extensively in phytotechnologies to clean sites contaminated with inorganic and organic constituents [5,6]. Moreover, given their greater productivity levels relative to most other temperate-grown, deciduous trees [7], hybrid poplars are ideal for providing biomass for multiple end uses (*i.e.*, the provisioning services).

Traditional uses of hybrid poplar biomass include fiber for the pulp and paper industry, as well as solid wood and chips for lumber and engineered wood products [8]. Hybrid poplar feedstocks are also critical components of current energy portfolios in North America [9–11], and have been used for energy in Europe for quite some time [12]. Hybrid poplar biomass is suitable for conversion to liquid transportation fuels via thermochemical and biochemical processes [13,14] and direct combustion during combined heat and power (CHP) operations [15]. Given the broad genetic variability within the genus *Populus* [16–18], certain genotypes exhibit wood property traits that are ideal for many of these uses, despite the fact that selection for one trait may reduce the quality and/or contribution of another. Physical traits that contribute to feedstock quality and, thus, the economic viability of growing SRWCs include but are not limited to: moisture content, bark:wood ratio, heating value, and specific gravity [19,20].

Specific gravity is often considered the most important feedstock characteristic for traditional products and energy applications [21–24]. For example, selection for hybrid poplar feedstocks with high densities and strengths may contribute to elevated pulp yields, while medium densities are more appropriate for oriented strand board (OSB) and plywood [8,25,26]. Likewise, high density wood is a requirement for thermochemical conversion and direct combustion technologies, whereby specific gravity and process efficiency are positively correlated [19,20]. Similarly, greater specific gravities increase transportation efficiencies because more biomass per truckload is delivered to processing facilities [22]. Overall, hybrid poplar specific gravity estimates reported in the literature (since 1979) range from 0.26 to 0.50 (Table 1).

Although yield has been the primary focus of most hybrid poplar tree improvement programs [8,27], the aforementioned benefits of specific gravity for multiple end uses have ensured it

has remained one of the key traits during breeding. One of the challenges during selection is the relationship between specific gravity and position along the bole of the tree, which has been somewhat inconsistent with tree age, site, and genotype [26,28–30]. Likewise, direct selection for specific gravity has at times been counterproductive to yield objectives given an inverse relationship between wood density and growth rate [23–25,28,31,32]. Although specific gravity of hybrid poplar feedstocks has been shown to be under strong genetic control [24,25,28,31,33], it is important to understand the role of genotype × environment interactions. Most specific gravity reports include numerous hybrid poplar genotypes but were conducted at a limited number of locations (*i.e.*, ≤4), resulting in a lack of information about the mechanisms and causes of specific gravity differences among genotypes tested across multiple sites differing in climate and soils.

Table 1. Summary of published studies testing specific gravity of *Populus*, since 1979.

Study Location	Age (yrs)	Genomic Group [1]	Clones (#)	Specific Gravity	Reference
Gorgan, Iran	22	D × N	1	0.33–0.36	Kord *et al.* [26]
Quebec, Canada	10, 12	D	4	0.37–0.39	Pliura *et al.* [24]
		T × D	3	0.34–0.36	
		D × N	4	0.32–0.34	
		M × B	4	0.29–0.31	
		B × N	4	0.31–0.34	
		Overall	19	0.29–0.45	
Quebec, Canada	10	D	3	0.36–0.37	Pliura *et al.* [23]
		T × D	3	0.33–0.34	
		D × N	3	0.32–0.35	
		M × B	3	0.28–0.32	
		Overall	12	0.26–0.38	
New York, USA	3	D × N	5	0.33–0.37	Tharakan *et al.* [22]
		N × M	2	0.34–0.36	
Quebec, Canada	3	Overall [2]	21	0.27–0.48	Zhang *et al.* [33]
Washington, USA	9	T	1	0.31–0.46	DeBell *et al.* [30]
		T × D	2	0.29–0.47	
Iowa, USA	4	A × A	1	0.32–0.42	Semen *et al.* [34]
		A × G	1	0.29–0.35	
		A × Tr	1	0.36–0.37	
Kansas, USA	4	D	9	0.34–0.40 [3]	Geyer *et al.* [29]
		D × N	2	0.35–0.37	
Quebec, Canada	9	D × N	10	0.30–0.40	Hernández *et al.* [32]
Hungary	10, 15	D × N	3	0.30–0.36	Mátyás and Peszlen [21]
Ontario, Canada	12	B	90	0.29–0.41	Ivkovich [31]
Quebec, Canada	9	D × N	10	0.28–0.41	Beaudoin *et al.* [28]
Pennsylvania, USA	4	M × T	1	0.39–0.46	Blankenhorn *et al.* [35]
Mississippi Valley, USA	3	D	75	0.27–0.39	Olson *et al.* [25]
Pennsylvania, USA	4	M	1	0.41–0.48	Murphey *et al.* [36]

Table 1. *Cont.*

Study Location	Age (yrs)	Genomic Group [1]	Clones (#)	Specific Gravity	Reference
		T	1	0.40–0.50	
		M × T	1	0.39–0.49	

[1] *Populus* species comprising the genomic groups were: (A) *P. alba* L.; (B) *P. balsamifera* L.; (D) *P. deltoides* Bartr. ex. Marsh; (G) *P. grandidentata* Michx.; (M) *P. maximowiczii* A. Henry (now considered a subspecies of *P. suaveolens* Fischer); (N) *P. nigra* L.; (T) *P. trichocarpa* Torr. & Gray; (Tr) *P. tremula* L; [2] A total of 21 clones were tested from the following genomic groups (individual values for each were not explicitly stated): D (1 clone); T × D (3); D × N (10); M × B (4); D × M (2); N × M (1); [3] 29 clones were tested; these values are from 11 clones reported in Table 5.

The overarching objective of the current study was to characterize specific gravity among hybrid poplar genotypes grown in Iowa, Minnesota, Wisconsin, and Michigan, USA. The plantation network consisted of ten genotypes grown at four sites for ten years, two genotypes grown at eleven sites for twenty years, and one genotype grown at two sites for fifteen years (Figure 1). Specific objectives for each set of plantations included: (i) testing for differences in specific gravity among three bole positions (diameter at breast height; 1/3 tree height; 2/3 tree height) to account for variability in age of the wood; (ii) testing for differences in specific gravity among genotypes, sites, and their interactions; and (iii) assessing the effects of climate and soils on specific gravity differences observed in (ii). These data are important for informing genotypic selection and silvicultural management decisions associated with growing hybrid poplars to increase ecosystem services across the landscape.

2. Experimental Section

2.1. Site and Clone Selection

Fifteen of the seventeen sites for the current study were selected from two regional networks of hybrid poplar clone and yield trials established in the north-central United States during 2000 to 2001 (10-year-old plantations) [27,37] and 1988 to 1991 (20-year-old plantations) [38–40]. The remaining two sites (15-year-old plantations) were established in 1995 as part of the woody biomass feedstock development program at Iowa State University (Figure 1). Table 2 highlights main characteristics for each site, including climate and soils information. Site latitude and longitude (decimal degrees) were determined from GPS coordinates taken at the sites. Soil texture, available soil water holding capacity (ASW; cm) for the top 100 cm, and depth to water table (WT; cm) were determined for each site using the Natural Resources Conservation Service (NRCS) Web Soil Survey [41]. Mean annual precipitation (P; mm) and mean growing season (April to October) temperature maximum (T_{max}; °C) and minimum (T_{min}; °C) were determined from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center using 30-year climate averages (1981 to 2010) from the weather station nearest to each site [42]. From T_{max} and T_{min}, average temperature (T_{avg}) and temperature differential (T_{diff}) were calculated ($T_{avg} = (T_{max} + T_{min})/2$; $T_{diff} = T_{max} - T_{min}$).

Figure 1. Plantation networks in a study testing site and genotype effects on specific gravity of hybrid poplars in the north-central region, USA.

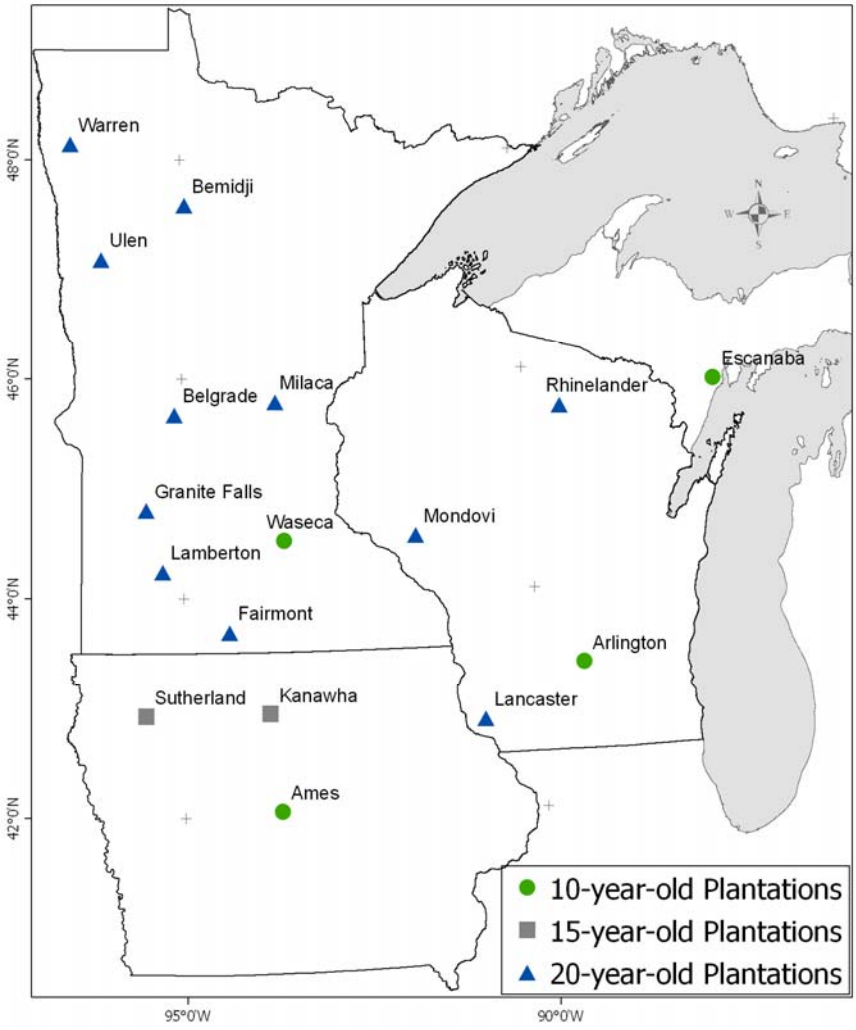


Table 2. Characteristics of plantations in a study testing site and genotype effects on specific gravity of hybrid poplars in the north-central region, USA.

State [1]	Site	Year Planted	Height (m) [2]	Lat (°N) [3]	Long (°W) [3]	Soil Texture	ASW (cm) [4]	WT (cm) [4]	*P* (mm) [5]	T_{avg} (°C) [5]	T_{diff} (°C) [5]
IA	Ames	2000	15.1 ± 0.4	42.05	93.66	Fine Sandy Loam	17.36	122	880.6	17.0	12.6
IA	Kanawha	1995	18.7 ± 0.3	42.93	93.80	Clay Loam	18.65	0	849.4	15.8	11.9
IA	Sutherland	1995	18.5 ± 0.6	42.93	95.54	Silty Clay Loam	20.40	0	780.3	16.3	13.9
MI	Escanaba	2001	12.5 ± 0.2	45.77	87.20	Fine Sandy Loam	15.01	>200	728.2	12.6	11.1
MN	Belgrade	1990	17.2 ± 0.4	45.67	95.11	Loam	18.20	76	653.0	15.3	14.3
MN	Bemidji	1988	17.9 ± 0.3	47.58	94.93	Loamy Sand	10.39	>200	676.4	12.7	11.8
MN	Fairmont	1988	18.7 ± 0.2	43.69	94.35	Clay Loam	18.35	15	830.8	16.5	11.4
MN	Granite Falls	1987	21.4 ± 0.8	44.80	95.52	Loam	19.09	75	726.7	15.3	12.8
MN	Lamberton	1988	18.7 ± 0.9	44.25	95.29	Clay Loam	18.00	15	709.7	15.6	13.3

Table 2. *Cont.*

State [1]	Site	Year Planted	Height (m) [2]	Lat (°N) [3]	Long (°W) [3]	Soil Texture	ASW (cm) [4]	WT (cm) [4]	*P* (mm) [5]	T_{avg} (°C) [5]	T_{diff} (°C) [5]
MN	Milaca	1989	19.4 ± 0.3	45.78	93.63	Silt Loam	18.39	15	748.3	14.1	12.9
MN	Ulen	1989	14.4 ± 0.5	47.09	96.18	Loam	16.90	15	628.1	14.3	13.2
MN	Warren	1989	20.8 ± 0.7	48.14	96.65	Fine Loamy Sand	9.73	60	547.6	13.4	14.3
MN	Waseca	2000	16.3 ± 0.4	44.06	93.54	Clay Loam	18.36	45	907.3	15.9	11.9
WI	Arlington	2000	18.0 ± 0.3	43.29	89.37	Silt Loam	21.64	61	869.2	14.7	13.9
WI	Lancaster	1991	22.5 ± 1.3	42.82	90.79	Silt Loam	15.23	>200	898.4	15.5	10.9
WI	Mondovi	1988	19.3 ± 0.3	44.52	91.65	Silt Loam	21.12	>200	881.1	15.4	15.0
WI	Rhinelander	1988	21.5 ± 0.6	45.63	89.46	Loamy Sand	7.43	61	675.4	13.0	12.9

[1] IA, Iowa; MI, Michigan; MN, Minnesota; WI, Wisconsin; [2] Mean height (± one standard error) of trees tested at each site (n = 4 to 39); [3] Lat, latitude; Long, longitude; [4] ASW, maximum available soil water for the top 100 cm; WT, depth to water table. Soil data (including texture) were obtained from the Natural Resources Conservation Service (NRCS) Web Soil Survey [41]; [5] *P*, mean annual precipitation; T_{avg}, mean growing season temperature (April to October); T_{diff}, difference between maximum and minimum growing season temperatures (T_{max} and T_{min}, not reported in the table). Climate data were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center using 30-year climate averages from 1981 to 2010 [42].

In total, twelve hybrid poplar clones belonging to five genomic groups were tested (Table 3). Ten clones were selected from the 10-year-old network based on availability at two or more of the four sites during initial surveys; however, some clones were not available at the time of harvest. Two clones were selected from the 20-year-old plantations based on tree health surveys conducted by Zalesny *et al.* [1]. The single hybrid aspen clone (*i.e.*, Crandon) from the 15-year-old sites was chosen based on current interest of assessing specific gravity of this genomic group for energy conversion and previous work by the research team on kraft pulp [34]. Table 3 also lists genomic groups and clones tested for each plantation network.

Table 3. Hybrid poplar genomic groups and clones sampled at each site in a study testing location and genotype effects on specific gravity in the north-central region, USA [1]. Site × clone combinations shaded in black were included in the original study but did not have requisite sample sizes for the current analyses.

Site	Clone											
	Crandon	DN182	DN34	C916000	C916400	C918001	NM2	NM6	NC13624	NC13649	NC13563	NC14018
	---------- *10-year-old plantations* ----------											
Ames												
Arlington												
Escanaba												
Waseca												

Table 3. *Cont.*

Site	*Clone*											
	Crandon	**DN182**	**DN34**	**C916000**	**C916400**	**C918001**	**NM2**	**NM6**	**NC13624**	**NC13649**	**NC13563**	**NC14018**
	---------- 15-year-old plantations ----------											
Kanawha												
Sutherland												
	---------- 20-year-old plantations ----------											
Belgrade												
Bemidji												
Fairmont												
Granite Falls												
Lamberton												
Lancaster												
Milaca												
Mondovi												
Rhinelander												
Ulen												
Warren												

Genomic Groups [2]

Populus deltoides

C916000, C916400, C918001

P. deltoides × *P. nigra*

DN34, DN182

P. alba × *P. grandidentata*

Crandon

P. nigra × *P. maximowiczii*

NM2, NM6

(*P. trichocarpa* × *P. deltoides*) × *P. deltoides*

NC13624, NC13649, NC13563, NC14018

[1] See Section 2.4 for a description of color designations; [2] See Table 1 for species authorities.

2.2. Field and Laboratory Sampling

In the field, a maximum of four dominant trees per clone were felled and total tree height was determined to the nearest 0.1 m at each site. Cross-sectional disks were harvested from each bole at 1.4-m height (*i.e.*, diameter at breast height, DBH), 1/3 height of the tree, and 2/3 height of the tree. Disks were sealed in plastic bags to avoid potential shrinkage, before transporting them for processing to the analytical laboratory at the Institute for Applied Ecosystem Studies in Rhinelander, WI, USA. Diameter to the nearest 0.1 cm and fresh mass to the nearest 0.1 g were determined for each disk. The disks were then oven-dried at 55 °C until constant mass, which was recorded with the same precision as fresh mass. One cross-sectional area of each disk was sanded, wetted, and imaged for ongoing studies. The sanded disks were then cut in half along a plane extending through the pith. A wedge, free of bark and defects, was cut from the half-disk beginning at the pith and extending to its outside edge.

2.3. Specific Gravity Measurements

Specific gravity was determined from each wedge according to the ratio between oven dry mass and green (saturated) volume. Specifically, each wedge was immersed in water for 24 to 36 h and placed into a water-filled desiccator under vacuum until they sank. After sinking, excess moisture was removed from all surfaces of each wedge, which was then placed into a beaker of water atop an analytical balance, and the displacement volume was determined to the nearest 0.1 g. Following green

volume determination, wedges were oven dried at 105 °C until constant mass, which was determined to the nearest 0.1 g.

2.4. Experimental Design and Data Analysis

Specific gravity data from 204 trees were assigned to one of three datasets: (i) 10-year-olds having a complete factorial design for sites and clones (depicted as light green with down-diagonal line in Table 3); (ii) 20-year-olds having a complete factorial design for sites and clones (depicted as dark green in Table 3); and (iii) a mixed dataset of 10-, 15-, and 20-year-olds having an incomplete factorial design (*i.e.*, lacking at least one site × clone combination required for a complete factorial of sites and clones; depicted as gray with up-diagonal line in Table 3). A summary of the datasets is shown in Table 4. For all datasets, only sufficiently represented clones (≥2 trees at a given site) were included in the analyses.

Table 4. Number of sites, clones, and site × clone combinations represented in the 10- and 20-year-old factorials, and mixed (10-, 15-, and 20-year-old) non-factorial analyses. Each site × clone combination was represented by two to four trees.

Dataset	Number of:			
	Trees	Sites [1]	Clones [1]	Site × Clone Combinations
10-year-old factorial	59	4	4	16
20-year-old factorial	71	9	2	18
Mixed (10-, 15-, and 20-year-old) non-factorial [2]	74	8	7	21

[1] See Table 3 for specific site × clone combinations; [2] For the mixed dataset, each clone was missing from 1 or more sites, and therefore no factorial analyses of site and clone were conducted. Thus, only differences between site × clone combinations were evaluated.

All three datasets were analyzed as split-plot designs. Site, clone and site × clone effects were evaluated at the main-plot level (with tree as the experimental unit), and bole position was evaluated at the split-plot level (with wedge as the experimental unit), for the 10-year-old and 20-year-old datasets. Site and clone were not included as treatment factors for the mixed dataset due to each clone being absent from at least one site; instead, the data were analyzed for differences among existing site × clone combinations at the main-plot level, with position being evaluated at the split-plot level. The data were analyzed using PROC MIXED (method = type 3) in SAS (SAS Institute, Inc., Cary, NC, USA), with denominator degrees of freedom calculated via the Kenwood-Rogers method [43]. When significant main effects and interactions were found ($p < 0.05$), multiple comparisons analyses with Tukey's adjustment [43] were conducted to identify significant differences ($p < 0.05$) among least-squares means. In the event a significant interaction containing a significant main effect was found, the interaction was evaluated rather than the main effect.

3. Results and Discussion

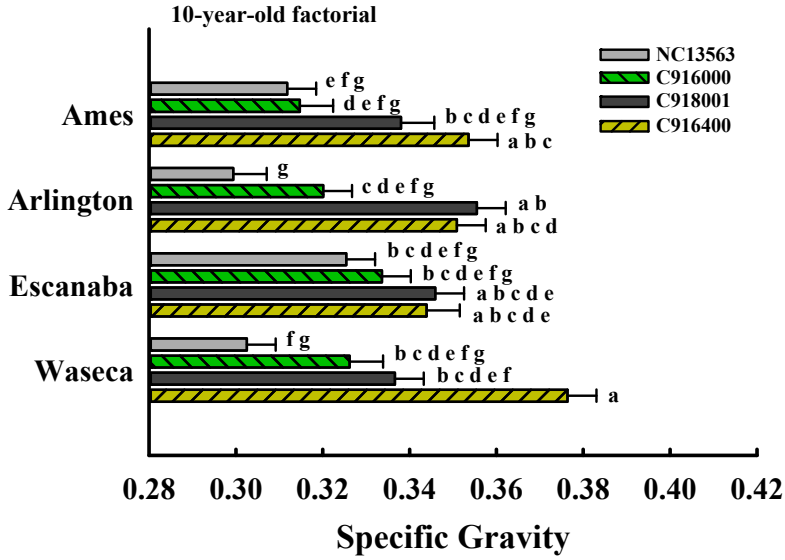
3.1. ANOVA Results

For the 10-year-old factorial, specific gravity was significantly influenced by position, clone, and site × clone interactions (Table 5). Multiple comparisons analysis of the position effect showed no significant difference between DBH (0.327 ± 0.003) and 1/3 height (0.329 ± 0.003), while 2/3 height was significantly higher than both of these (0.344 ± 0.003). Multiple comparisons analysis of site × clone effects showed the specific gravities for clones NC13563 and C916000 were consistently lowest and second-lowest, respectively, across all sites (Figure 2). Clone C918001 had the highest specific gravity at Escanaba and Arlington (where it was significantly higher than NC13563 and C916000), while C916400 was highest at Ames (where it was significantly higher than NC13563 and C916000) and Waseca (where it was significantly higher than all the other clones).

Table 5. Probability values from analyses of variance comparing specific gravity of hybrid poplar clones grown at 17 sites throughout the north-central region, USA. Three bole positions were also tested. See Experimental Section for descriptions of all fixed effects. Significant values are in bold.

Source of Variation	*p*-Value
------- *10-year-old factorial* -------	
Site	0.4011
Clone	**<0.0001**
Site × Clone	**0.0078**
Position	**0.0002**
Position × Site	0.1293
Position × Clone	0.5963
Position × Site × Clone	0.5212
------- *20-year-old factorial* -------	
Site	**<0.0001**
Clone	0.4529
Site × Clone	**0.0430**
Position	**<0.0001**
Position × Site	**0.0010**
Position × Clone	**0.0183**
Position × Site × Clone	0.1918
------- *Mixed (non-factorial)* -------	
Site × Clone	**<0.0001**
Position	**<0.0001**
Position × Site × Clone	0.1213

Figure 2. Specific gravity of four hybrid poplar clones grown at four sites in the north-central region, USA. Each bar represents the mean of two to four trees with one standard error, according to the factorial analysis of 10-year-old plantations. Bars labeled with different letters are different at $p < 0.05$.



For the 20-year-old factorial, specific gravity was significantly influenced by position, position × clone, position × site, site, and site × clone (Table 5). Multiple comparisons analysis of the position × clone effect showed all positions were significantly different for DN34 and increased from DBH (0.353 ± 0.003) to 1/3 height (0.364 ± 0.003) to 2/3 height (0.385 ± 0.003). Similarly, all positions were significantly different for DN182 and increased from DBH (0.346 ± 0.003) to 1/3 height (0.367 ± 0.003) to 2/3 height (0.396 ± 0.003). The differences between the two clones were not significant at DBH or 1/3 height, but were significant at 2/3 height. For the position × site effect, multiple comparisons analysis showed that specific gravity at 2/3 height was significantly greater than DBH (with 1/3 height being intermediate) for eight of the nine sites (Table 6). The nature of the intermediate specific gravities at 1/3 height varied by site and can be summarized as follows: (i) significantly lower than at 2/3 height but not significantly different than at DBH (Bemidji, Granite Falls, Mondovi, Rhinelander); (ii) not significantly different than at 2/3 height but significantly higher than at DBH (Fairmont, Lamberton); (iii) significantly lower than at 2/3 height while also significantly higher than at DBH (Belgrade, Milaca). One site showed no significant position effects (Warren). Multiple comparisons analysis of site × clone effects showed the specific gravities for clones DN34 and DN182 at Mondovi were significantly lower than DN34 at Belgrade and DN182 at Fairmont and Lamberton (Figure 3). Clones DN182 at Granite Falls and DN34 at Rhinelander were also significantly lower than DN182 at Fairmont.

For the mixed non-factorial analysis, specific gravity was significantly influenced by position and site × clone interactions (Table 5). Multiple comparisons analysis of the position effect showed no significant difference between DBH (0.335 ± 0.003) and 1/3 height (0.338 ± 0.003), with 2/3 height being significantly higher than both of these (0.366 ± 0.003). For site × clone effects, multiple comparisons analysis showed a general trend of Crandon and DN34 having the highest specific

gravities across available sites, with NC13649 and NC13624 exhibiting the lowest specific gravities, and the remaining clones (NC14018, NM2, and NM6) being intermediate (Figure 4).

Table 6. Least-squares means of specific gravity (± one standard error) for each combination of site and bole position from the 20-year-old factorial analysis of variance in a study testing site and genotype effects on specific gravity of hybrid poplars in the north-central region, USA. Combinations with different letters are significantly different at $p < 0.05$.

Site	Position [1]	Specific Gravity
Belgrade	2/3	0.412 ± 0.007 a
Fairmont	2/3	0.405 ± 0.007 ab
Milaca	2/3	0.400 ± 0.007 abc
Fairmont	1/3	0.397 ± 0.007 abcd
Bemidji	2/3	0.396 ± 0.007 abcde
Rhinelander	2/3	0.395 ± 0.007 abcde
Lamberton	2/3	0.387 ± 0.007 bcdef
Granite Falls	2/3	0.380 ± 0.008 cdefg
Lamberton	1/3	0.379 ± 0.007 defg
Warren	2/3	0.377 ± 0.007 efgh
Warren	DBH	0.372 ± 0.007 fghi
Belgrade	1/3	0.371 ± 0.007 fghi
Milaca	1/3	0.370 ± 0.007 fghij
Warren	1/3	0.366 ± 0.007 ghijk
Mondovi	2/3	0.364 ± 0.007 ghijkl
Bemidji	1/3	0.362 ± 0.007 ghijklm
Lamberton	DBH	0.359 ± 0.007 hijklmn
Fairmont	DBH	0.358 ± 0.007 hijklmn
Granite Falls	1/3	0.353 ± 0.008 ijklmno
Rhinelander	1/3	0.353 ± 0.007 ijklmno
Bemidji	DBH	0.351 ± 0.007 jklmno
Belgrade	DBH	0.346 ± 0.007 klmno
Milaca	DBH	0.345 ± 0.007 lmno
Granite Falls	DBH	0.342 ± 0.008 mno
Rhinelander	DBH	0.340 ± 0.007 no
Mondovi	1/3	0.339 ± 0.007 no
Mondovi	DBH	0.333 ± 0.007 o

[1] DBH, diameter at breast height (1.4 m); 1/3, one-third height of the tree; 2/3, two-thirds height of the tree.

Figure 3. Specific gravity of hybrid poplar clones DN34 and DN182 grown at nine sites in the north-central region, USA. Each bar represents the mean of two to four trees with one standard error, according to the factorial analysis of 20-year-old plantations. Bars labeled with different letters are different at $p < 0.05$.

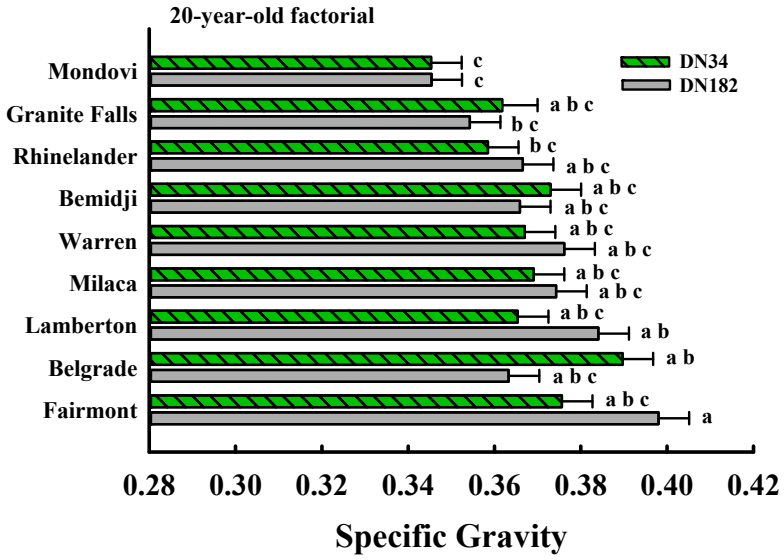
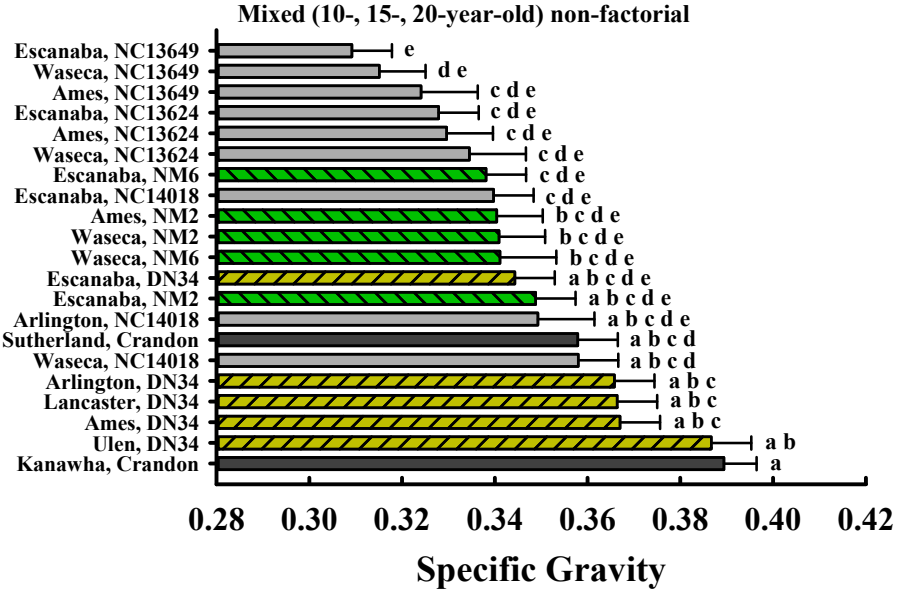


Figure 4. Specific gravity of seven hybrid poplar clones grown at eight sites in the north-central region, USA. Each bar represents the mean of two to four trees with one standard error, according to the mixed (nested) analysis of 10-, 15-, and 20-year-old plantations. Bars labeled with different letters are different at $p < 0.05$. Bars are shaded according to genomic group, as defined in Table 3.



3.2. Interpretation of Position Effects

The effects of bole position on specific gravity vary widely in the literature, likely due in part to differences in the relative bole positions sampled in different studies. However, some general observations can be made. For example, a trend of specific gravity decreasing with increasing height

has been reported [26]. Others have reported decreasing specific gravity in mid-heights but similar densities at low and high bole positions [28–30]. Geyer *et al.* [29] found that branch wood (analogous to the 2/3 height position in this study) had significantly higher specific gravity than DBH wood, which corroborates the general trend observed for all three datasets in this study. Specifically, the 10-year-old and mixed datasets showed significantly higher specific gravities at 2/3 height than at DBH across all sites and clones, and the position × site effect for the 20-year-old dataset showed the same trend at eight of the nine sites evaluated. Previous research with hybrid poplars found that wood near the pith had higher specific gravity than subsequent growth rings [28]. Thus, the higher proportion of near-pith wood at 2/3 height (relative to that at DBH) likely explains the higher specific gravities observed in this study.

3.3. Interpretation of Site × Clone Interactions Using Site Covariates

To better understand the significant site × clone effects observed in this study, readily-available site variables were evaluated as covariates for the least-squares means of specific gravity: latitude (L), available soil water holding capacity (ASW), water table depth (WT), annual precipitation (P), growing season average temperature (T_{avg}), and growing season temperature differential (T_{diff}); see Table 2 for descriptions and values. In addition, mean growth rate (cm·year^{-1}) based on outside bark diameter (OBD) at breast height for each clone at each site was evaluated as a covariate. Using PROC GLM in SAS, the effects of clone, each covariate, and their interactions were analyzed for statistical significance across all 204 trees from all three datasets. Non-significant factors were removed in a stepwise fashion until only significant factors ($p < 0.05$) remained. The resulting model indicated significant effects of clone ($p < 0.0001$), T_{diff} ($p = 0.0272$), and clone × WT ($p = 0.0494$) on specific gravity, as well as a strong model fit ($R^2 = 0.89$).

The coefficient for T_{diff} was negative (−0.00366), indicating that the trees produced wood of lower specific gravity under higher temperature differentials. This is consistent with previous research showing lower specific gravities of wood produced under sub-optimal conditions [24] and particularly conditions of water stress [44], presumably due to reduced cell wall thickness. This water stress hypothesis is further supported by analysis of the slopes associated with the clone × WT interaction (Table 7). For clones C916400, DN182, and DN34 (10-year-olds) the slopes are all negative and significantly different from 0, which indicates that deeper water tables (and presumably greater water stress) are associated with lower specific gravities for these clones. For the remaining clones, the slopes were found to be not significantly different from 0, meaning the clones were not significantly affected by water table depth. Interestingly, the slope for the 10-year-old DN34 was significantly different from 0, whereas that for the 20-year-old DN34 was not. This suggests that the effects of WT on some clones may decrease with age, possibly as the trees grow larger and develop root systems capable of reaching deeper water tables. Thus, genetic and/or developmental differences in rooting depth may explain why some clones appear more sensitive to WT than others. However, further study is recommended to specifically test these hypotheses.

Table 7. Probability values from analyses of variance testing the hypothesis that slopes are equal to zero for the clone × depth to water table (WT) interaction, by clone, in a study testing site and genotype effects on specific gravity of hybrid poplars in the north-central region, USA. Significant values are in bold.

Clone	Age (y)	$\text{Slope}_{(\text{Clone} \times \text{WT})}$	$P_{(\text{Slope} = 0)}$
C916000	10	0.000015	0.8636
C916400	10	−0.000186	**0.0394**
C918001	10	−0.000038	0.6669
DN34	10	−0.000234	**0.0411**
DN34	20	−0.000070	0.1084
DN182	20	−0.000138	**0.0105**
NC13563	10	0.000124	0.1625
NC13624	10	−0.000062	0.5159
NC13649	10	−0.000058	0.5440
NC14018	10	−0.000139	0.1216
NM2	10	0.000031	0.7426
NM6	10	−0.000039	0.6836

In the ANOVA of the 10-year-old dataset (Figure 2), the effects of T_{diff}, and clone × WT are apparent when comparing C916400 (a WT-sensitive genotype) with the remaining (non-WT-sensitive) clones. The non-WT-sensitive clones had their highest (NC13563, C916000) or second-highest (C918001) specific gravities at Escanaba, which had the lowest T_{diff} (11.1 °C) of the 10-year-old sites (Table 2). In contrast, the WT-sensitive clone C916400 had its highest specific gravities at Waseca (which had the shallowest WT at 45 cm) and its lowest values at Escanaba (which had the deepest WT at >200 cm). Similarly, in the ANOVA of the 20-year-old dataset (Figure 3), two of the three highest specific gravities were observed for DN182 (a WT-sensitive genotype) at Fairmont and Lamberton (tied for shallowest WT at 15 cm). The lowest specific gravities were observed for DN182 and DN34 at Mondovi, which had both the deepest WT (tied with Bemidji at >200 cm) and the highest T_{diff} (15.0 °C). For the mixed dataset, the only WT-sensitive genotype (10-year-old DN34) had lower specific gravities at Escanaba (WT >200 cm) than at Arlington (WT = 61 cm) and Ames (WT = 122 cm).

3.4. Correlations between Specific Gravity and Growth Rate

One of the most challenging aspects of selection for specific gravity is its negative relationship with growth rate, with correlations ranging from −0.66 to −0.28 [23–25,28,31,32]; however, decades of breeding for improved genotypes have resulted in some progress towards closing this gap. For example, DeBell *et al.* [30] reported growth rate did not influence wood density for three hybrid poplar clones in western Washington, USA. Likewise, Zhang *et al.* [33] reported wood density was not significantly correlated with growth for 21 hybrid poplar clones at two sites in southern Quebec, Canada. As alluded to above, OBD growth rate was not found to be a significant covariate for specific gravity in this study. Similarly, the correlation between OBD and specific gravity was found to be weak (though statistically significant) across all datasets ($r = -0.41$; $p < 0.0001$), as well as for individual analyses: (i) 10-year-old dataset ($r = -0.24$; $p = 0.0016$); (ii) 20-year-old dataset ($r = -0.35$; $p < 0.0001$); (iii) mixed dataset ($r = -0.34$; $p < 0.0001$). The weak nature of this relationship may

logically be attributable to some of the factors which influence growth rate having opposing effects on specific gravity. For instance, both decreased water stress and increased nitrogen availability would be expected to increase growth rate; however, the former would be expected to result in higher specific gravities (as demonstrated in this study) and the latter would be expected to result in lower specific gravities (as demonstrated by Pitre *et al.* [45] and Hacke *et al.* [46]). Thus, the specific factors dictating growth rate at a given site (e.g., climate, soils, nutrient management, *etc.*) should be expected to be more informative for explaining differences in specific gravities than growth rate itself.

3.5. Genetic Trends in Specific Gravity

In general, genetic control of specific gravity was moderate to high in the reported literature, with broad sense heritabilities or repeatabilities ranging from 0.45 to 0.92 [24,25,28,31,33]. More specifically, common trends existed across taxonomic sections and genomic groups, with *Aigeiros* genotypes (*P. deltoides* and *P. nigra* parentage) exhibiting similar densities to those belonging to the *Populus* section (*P. alba*, *P. grandidentata*, *P. tremula*), which were both approximately 9% less than the *Tacamahaca* species (*P. balsamifera*, *P. trichocarpa*, *P. maximowiczii*) (Table 1). One exception was reported at the species level [23,24], whereby genomic groups with *P. balsamifera* parentage exhibited uncharacteristically low maximum specific gravities (≤0.32) relative to their *Tacamahaca* counterparts (≤0.42). Although the design of the current study did not warrant heritability estimation or specific testing of these observations, trends associating specific gravity with taxonomic sections and genomic groups existed. In contrast to previous studies, our genotypes with *Tacamahaca* parentage exhibited the lowest overall specific gravities (~0.359), while *Aigeiros* (~0.372) and *Populus* (~0.386) differed observationally from *Tacamahaca* and one another. These results are likely due to the fact that the number of genotypes per genomic group was limited in the current study. For example, the high ranking of the *Populus* section was attributed to one clone, Crandon (*P. alba* × *P. grandidentata*). Other hybrids within the section *Populus* have exhibited much lower densities [34]. In addition, the *Tacamahaca* genotypes tested were not ideal for these comparisons. The first two clones, NM2 and NM6, were typically the most productive in the north-central region for traditional applications [27,37]; therefore, the negative correlation between specific gravity and growth rate reported above may have accounted for the majority of the responses observed (*i.e.*, fast growth contributed to low density wood). The second *Tacamahaca* genomic group ((*P. trichocarpa* × *P. deltoides*) × *P. deltoids*) contained, at most, 25% of its alleles from *P. trichocarpa*, which may not be a true representation of the species as reported elsewhere [30,36]. Nevertheless, given the use of molecular genetics technologies, detailed studies with requisite designs for testing these relationships would be very beneficial for hybrid poplar production systems.

4. Conclusions

Specific gravity of individual trees in the current study ranged from 0.267 to 0.495, with a mean of 0.352 ± 0.001 (n = 612 samples from 204 trees). These values corroborated previous reports for hybrid poplars (Table 1). The variation in specific gravity in the current study was primarily attributed to three factors. First, within-tree variability associated with bole position from which stem disks were harvested showed that specific gravity increased with increasing tree heights. Second, genotype × environment

interactions significantly influenced specific gravity for 10-, 15-, and 20-year-old plantations. In particular, site characteristics associated with increasing water stress resulted in decreased specific gravities. The two most important factors controlling this response were depth to water table and the differential between mean maximum and minimum temperature for the growing season (April to October). Third, specific gravity was tightly related to taxonomic sections, species, and genomic groups. In contrast to previous studies, however, *Tacamahaca* genotypes exhibited lower densities relative to *Aigeiros* or *Populus*, with the latter having the highest specific gravities. Trees in the current study also exhibited a weak, inverse relationship between specific gravity and growth rate. Overall, these results are important for informing tree improvement decisions related to the choice of parental material and subsequent selection for yield versus wood quality traits, especially for provisioning ecosystem services such as woody biomass for pulp and paper, solid wood products, and biofuels/bioenergy.

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Conflict of Interest

The authors declare no conflict of interest.

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